Observations on the Structure, Derivation and Nature of Melanin.

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Developments in enzyme and cellular chemistry have clearly demonstrated that the nucleus is the center of metabolism of nucleoproteins. The most intense production of nucleoprotein appears to take place in the nucleolus, and the material diffuses to, and through, the nuclear membrane into the cytoplasm. As shown by DuBuy and his coworkers, the melanin of the S91 melanoma contains approximately 20% nucleic acids. This would lead to the suspicion that melanin is closely allied to, or identical with, nucleoproteins, especially when the morphological picture is taken into account.

A series of color slides of pertinent cells will be presented, enlarged 540 times, which give a pictorial representation of the following pattern: Melanin first appears in the nucleolus and spreads to the linin framework of the nucleus. It appears in intranuclear vacuoles and finally involves the entire nucleus and the membrane. It leaves the nucleus by way of the chromidial derivatives.

We are of the opinion that this demonstration clearly indicates that the source of melanin formation is within the nuclear material. It appears that the life history of the melanin—as demonstrated—closely parallels that of chromatin and that nucleoprotein is replaced by melanoprotein.

It can be noted from the pictures to be presented that melanized nuclei show a considerable variation in size, by measurement, from 7×7 micra up to 16×36 micra. This raises the question as to whether these larger nuclei result from further growth of the melanized chromatin, or from propagation of individual melanin granules.

Their main features, so far observed, include the characteristics of membranes, an inner body showing amitotic division; "X" bodies which could not be identified, which, however, are an apparatus of propagation, connected with the process of budding and of buckling of the membrane. This process leads to buds connected with the mother-body by fine strands or a solid stem. These buds also show the "X" structure and may undergo severance from the mother body; free buds which no longer can be differentiated from melanin granules; and cloudy masses spreading from melanin granules in which new granules make their appearance.

In general, the youngest buds are colorless and show all transitions to deep coloring, just as described by Smith in his cultures of the pigmented epithelium of the eye. We have, on occasion, succeeded in differentially staining the body and the bud by neutral red as a basic dye and light green as an acid dye. The buds have been seen to stain acidophilic with light green. Supravital staining with borax-methylene blue applied to unfixed specimens by Scott's method often shows the young buds to be colorless. These young buds appear to be without a membrane and as they develop, they stain blue and a membrane makes its appearance.

The most striking feature of all of these buds is the presence of the "X" structure. Entirely the same buds with "X" structures are also found, in very great numbers, separated from melanin granules freely in the specimen.

While further work is warranted, in order to understand the foregoing observations in their entirety, we believe that the outstanding features of melanin granules in melanomas and mammalian choroids are compatible with those of a living, dividing, and budding structure derived from living, dividing and budding nuclear material.

References on the Main Investigations of the Relationship: Chromatin and Melanin.

A. Nucleolus and Melanin.

Roessle, R. Der Pigmentierungsvorgang im Melanosarcom. *Ztschr. Krebsforsch.*, 2:291-332, 1904.

"The most remarkable observation is the extrusion of nucleolar material into the cytoplasm and its transformation into melanin."

Meirowsky, E. Die Entstehung des Oberhautpigments aus der Substanz der Kernkoerperchen. *Monatshefte Prakt. Derm.* 43:155-163, 1906.

———— Ueber den Ursprung des Melanotischen Pigments der Haut und des Auges. Publ. W. Klinkhardt, Leipzig, 1908.

"Formation of pigment is preceded by an augmentation of nucleolar material irrespective of the material under observation (skin of man and rabbits after irradiation, embryonic skin, triton larves, pigment of the eye, nevi melanomas). The nucleolar material is transformed into melanin."

Goda, T. Cytoplasmic Inclusions of Amphibian Cells with Special Reference to Melanin Formation. *J. Faculty of Science Imperial University, Tokyo.* Section IV, Zoology, First Part, Vol. II, 51-122, 1928-1931.

"The melanin granules arise from the nucleus by the following process: nucleolar bodies — nucleoliquid drops — melanogranules."

Schultz, O. T. The Formation of Pigment by the Dermal Chromatophores. *J. Med. Res.*, 26 (new series 21): 65-77, 1912.

"... chromatin is thrown out of the nucleus into the cytoplasm, leading to the formation of a functional chromatin net. The chromatin present in the cytoplasm becomes changed into a material which has the staining reactions of nucleolar substance. Further change leads to the formation of this material into pigment."

"(Pigment) ... is the product of the specialized physiological activity of the cell by which nuclear derivates are transformed directly into pigment."

B. Melanization of Nuclei, Chromidia and Mitoses.

VON SZILY, A. Ueber die Entstehung des Melanotischen Pigmentes im Auge der Wirbeltierembryonen und im Choroidealsarkomen. *Arch. Mikr. Anat.*, 77: 1-70, 1911.

"The colorless stromata of the melanin of metazoa originate in all cases examined exclusively in the nucleus. Their direct origin from the chromatin of the nucleus and their transformation into the cytoplasm can exactly be followed up. These 'chromidia' change over into melanin under the influence of ferments."

JELIASKOWA PASPALEWA, A. Cytologische Untersuchungen ueber die Entstehung des Melanotischen Pigments. *Ztschr. Wissenschaftl. Zoologie*, 137: 365-402, 1930.

"The observation of a total melanization of chromosomes makes it impossible to contest the origin of melanin from chromatin."

LUDFORD, R. J. Nuclear Activity during Melanosis with Special Reference to Melanin Formation in a Melanotic Sarcoma. *J. Royal Micr. Soc.*, 13-28, 1924.

———— The General and Experimental Cytology of Cancer. *J. Micr. Soc.*, 249-292, Sept. 1925.

"Melanosis occurring during mitosis and following chromatin extrusion, nuclear fragmentation, nuclear budding, karrhyorrhexis, and pycnosis." Ludford believes that the greatest part of the melanin is formed in the cytoplasm under the influence of the Golgi apparatus which is concerned with enzyme formation.

C. Melanization of Intranuclear Vacuoles.

MEIROWSKY, E. Die Entstehung des Oberhautpigments aus der Substanz der Kernkoerperchen. *Montsh. Prakt. Derm.*, 43: 155-163, 1906.

"A cavity is formed in the nucleus with melanin granules at its wall. Through the ruptured wall pigment is extruded into the protoplasm."

LUDFORD, R. J. Nuclear Activity During Melanosis with Special Reference to Melanin Formation in a Melanotic Sarcoma. *J. Roy. Micr. Soc.*, 13-28, 1924.

Summary 8. "Melanin is often formed inside the nucleus, generally in intranuclear vacuoles."

APITZ, K. Ueber die Pigmentbildung in den Zellkernen Melanotischer Geschwueslte. *Virchow Arch.*, 300: 89-112, 1937.

"The nucleus forms the mother substance of melanin." "The origin of the nuclear melanin takes place within vacuoles owing to the retention of physiological secretion product of the nucleus."

D. Biochemistry of Nucleoproteins.

DEROBERTIS, E. D. P., NOWINSKI, W. M. AND SAEZ, F. A. General Cytology. Publ. W. B. Saunders, Philadelphia and London, 1938.

E. Budding Processes in Individual Melanin Granules.

As far as known, no references are available.

Evidence for the Mitochrondrial Nature and Function of Melanin Granules.

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Melanin occurs in the cytoplasmic granules of the melanoblasts of the Harding-Passey and Cloudman S91 mouse melanomas, and also, of course, in the phagocytes of these tumors. In both tumors, cells occur in which all, or nearly all, of the visible cytoplasmic granules are melanized. Colorless granules of similar size occur in amelanotic or partially amelanotic cells of the S91 tumor and also in the derived Algire partially amelanotic S91A melanoma. Some colorless granules usually occur in the perikaryon of the Harding-Passey melanoblast. The S91A amelanotic melanoma sometimes contains cells in which the cytoplasmic granules are very slightly melanized.

The cytoplasmic granules (melanized and non-melanized) are the only structures which stain with Janus Green B in the absence of nuclear staining by this dye. The granule staining is reversibly dependent upon oxygen tension. No other structures resembling mitochondria are visible in the cells. On alkaline hydrolysis, both melanized and non-melanized granules yield solutions with strong ultraviolet absorption at 2580A, and contain organic phosphorus and pentose. Centrifugally isolated cytoplasmic granules of amelanotic melanoma cells possess enzymic activities characteristic of mitochondria of other origin (e.g., from liver, kidney, heart). Aerobically, these activities include the cytochrome oxidase and succinic oxidase systems. Anaerobically, the cytoplasmic granules possess glycolytic activities also comparable to those of typical mitochondria. Centrifugally isolated melanized granules from both Harding-Passey and S91 melanomas possess not only all of the enzymic activities found in the amelanotic granules, but also dopa oxidase activity. On the basis of the foregoing morphologic, chemical and enzymic data it is concluded that the melanized and non-melanized granules of these mouse melanomas are mitochondria.

Mitochondria are fundamental structures in both animal and plant cells, and in plants it is now well established that they derive from pre-existing mitochondria, possess a complex hereditary system (chondriogenes), and are capable of mutation. In mutant states plant mitochondria display abnormal enzymic activities. Self-duplication of these abnormal mitochondria results in development of neoplasia. Plant mitochondria may be specifically modified by certain viruses that result in neoplasias very similar to those caused by mutant chondriogenes. The behavior of normal, mutant or virus-modified mitochondria may also be affected by specific nuclear genes.